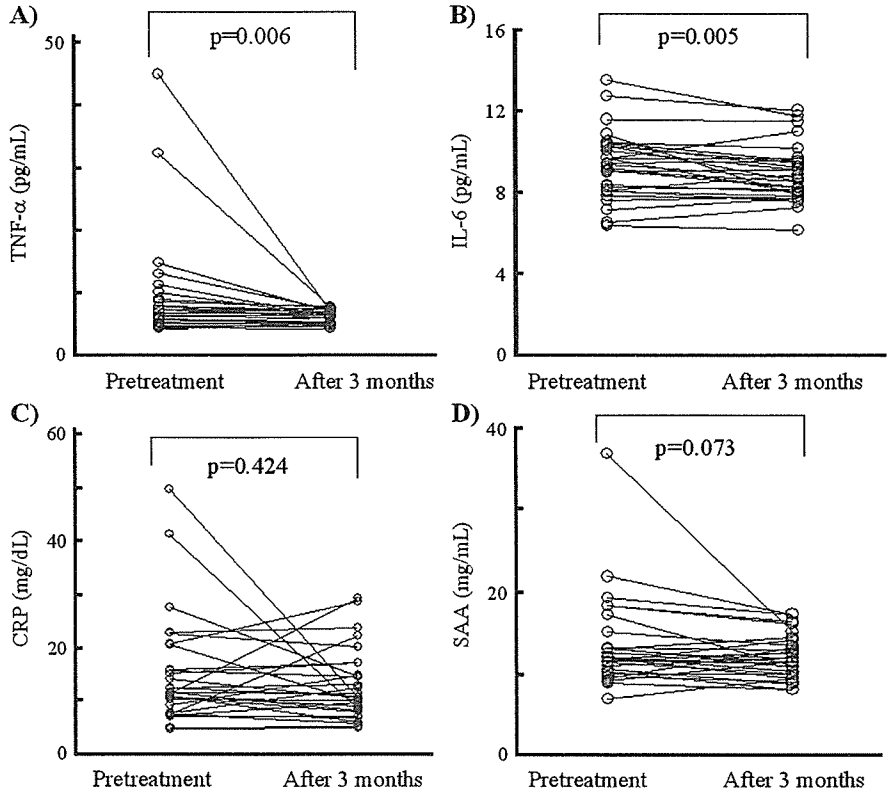


FIGURE 2. Effects of valsartan on serum tumor necrosis factor-α, interlukin-6, C-reactive protein, and serum amyloid A. Three months of treatment with valsartan significantly reduced serum tumor necrosis factor-α, interlukin-6, but not C-reactive protein and serum amyloid A.

the mechanisms responsible for the suppression of new-onset diabetes mellitus.

There are several limitations in the present study. First, a small number of subjects were enrolled. Since renal dysfunction, diabetes mellitus, thyroid disease, acute inflammatory diseases, and malignant disease have influences of serum pro-inflammatory cytokines, patients with these diseases are excluded from the study. Second, this is not a randomized, blinded study and does not include a control group of healthy volunteers. Third, after 3 months of treatment with 40 to 80 mg valsartan, both serum CRP and SAA tended to decrease. We could not show the effects of optimal dose of valsartan for longer periods on CRP and SAA levels in current study. Further study is needed to address these limitations.

In conclusion, the ARB, valsartan, reduced blood pressure and serum pro-inflammatory cytokines, TNF-α, and IL-6 in patients with essential hypertension. These results indicate that valsartan reduces vascular wall inflammation and further cardiovascular events in essential hypertensive patients.

REFERENCES

1. Lacy F, O'Connor DT, Schmid-Schonbein GW. Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. *J Hypertens.* 1998;16:291–303.
2. Bautista LE, Vera LM, Arenas IA, et al. Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF-alpha) and essential hypertension. *J Hum Hypertens.* 2005;19:149–154.
3. Chae CU, Lee RT, Rifai N, et al. Blood pressure and inflammation in apparently healthy men. *Hypertension.* 2001;38:399–403.
4. Bautista LE, Lopez-Jaramillo P, Vera LM, et al. Is C-reactive protein an independent risk factor for essential hypertension? *J Hypertens.* 2001;19: 857–861.
5. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res.* 2001;89:763–771.
6. Engstrom G, Janzon L, Berglund G, et al. Blood pressure increase and incidence of hypertension in relation to inflammation-sensitive plasma proteins. *Arterioscler Thromb Vasc Biol.* 2002;22:2054–2058.
7. Rus HG, Niculescu F, Vlaicu R. Tumor necrosis factor-alpha in human arterial wall with atherosclerosis. *Atherosclerosis.* 1991;89:247–254.
8. Rus HG, Vlaicu R, Niculescu F. Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. *Atherosclerosis.* 1996;127:263–271.
9. Hatanaka K, Li XA, Masuda K, et al. Immunohistochemical localization of C-reactive protein-binding sites in human atherosclerotic aortic lesions by a modified streptavidin-biotin-staining method. *Pathol Int.* 1995;45: 635–641.
10. Feingold KR, Serio MK, Adi S, et al. Tumor necrosis factor stimulates hepatic lipid synthesis and secretion. *Endocrinology.* 1989;124:2336–2342.
11. Hotamisligil GS, Spiegelman BM. Tumor necrosis factor alpha: a key component of the obesity-diabetes link. *Diabetes.* 1994;43:1271–1278.
12. Ferrari R. The role of TNF in cardiovascular disease. *Pharmacol Res.* 1999;40:97–105.
13. Ruiz-Ortega M, Ruperez LM, Lorenzo O, et al. Role of renin-angiotensin system in vascular diseases: expanding the field. *Hypertension.* 2001;38: 1382–1387.

14. Brasier AR, Recinos A III, Eledrisi MS. Vascular inflammation and the renin-angiotensin system. *Arterioscler Thromb Vasc Biol.* 2002;22:1257–1266.
15. Dahlof B, Devereux RB, Kjeldsen SE, et al. Cardiovascular morbidity and mortality in patients with diabetes in the losartan intervention for endpoint reduction in hypertension study (LIFE): a randomized trial against atenolol. *Lancet.* 2002;359:995–1003.
16. von zur Muhlen B, Kahan T, Hagg A, et al. Treatment with irbesartan or atenolol improves endothelial function in essential hypertension. *J Hypertens.* 2001;19:1813–1818.
17. Kubo A, Fukuda N, Soma M, et al. Inhibitory effect of angiotensin II type 1 receptor antagonist on growth of vascular smooth muscle cells from spontaneously hypertensive rats. *J Cardiovasc Pharmacol.* 1996;27:58–63.
18. Fliser D, Buchholz K, Haller H. For the European trial on olmesartan and pravastatin in inflammation and atherosclerosis (EUTOPIA) Investigators. Antiinflammatory effects of angiotensin II subtype 1 receptor blockade in hypertensive patients with microinflammation. *Circulation.* 2004;110: 1103–1107.
19. Pai JK, Pischon T, Ma J, et al. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med.* 2004;351:2599–2610.
20. Yasunari K, Maeda K, Watanabe T, et al. Comparative effects of valsartan versus amlodipine on left ventricular mass and reactive oxygen species formation by monocytes in hypertensive patients with left ventricular hypertrophy. *J Am Coll Cardiol.* 2004;43:2116–2123.
21. Navalkar S, Parthasarathy S, Santanam N, et al. Irbesartan, an angiotensin type 1 receptor inhibitor, regulates markers of inflammation in patients with premature atherosclerosis. *J Am Coll Cardiol.* 2001;37:440–444.
22. Prasad A, Koh KK, Schenke WH, et al. Role of angiotensin II type 1 receptor in the regulation of cellular adhesion molecules in atherosclerosis. *Am Heart J.* 2001;142:248–253.
23. Chabrashvili T, Kitiyakara C, Blau J, et al. Effects of ANG II type 1 and 2 receptors on oxidative stress, renal NADPH oxidase, and SOD expression. *Am J Physiol Regul Integr Comp Physiol.* 2003;285:R117–R124.
24. Dandona P, Kumar V, Aljada A, et al. Angiotensin II receptor blocker valsartan suppresses reactive oxygen species generation in leukocytes, nuclear factor-κB, in mononuclear cells of normal subjects: evidence of an antiinflammatory action. *J Clin Endocrinol Metab.* 2003;88:4496–4501.
25. Carey RM, Jin XH, Siragy HM. Role of the angiotensin AT2 receptor in blood pressure regulation and therapeutic implications. *Am J Hypertens.* 2001;14:98S–102S.
26. Criscione L, de Gasparo M, Buhlmayer P, et al. Pharmacological profile of valsartan: a potent, orally active, nonpeptide antagonist of the angiotensin II AT1-receptor subtype. *Br J Pharmacol.* 1993;110:761–771.
27. Siragy HM. Angiotensin receptor blockers: how important is selectivity? *Am J Hypertens.* 2002;15:1006–1014.
28. Tsutamoto T, Wada A, Maeda K, et al. Angiotensin II type 1 receptor antagonist decreases plasma levels of tumor necrosis factor alpha, interleukin-6 and soluble adhesion molecules in patients with chronic heart failure. *J Am Coll Cardiol.* 2000;35:714–721.
29. Furuhashi M, Ura N, Higashiura K, et al. Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. *Hypertension.* 2003;42:76–81.
30. Kem PA, Saghizadeh M, Ong JM, et al. The expression of tumor necrosis factor in human adipose tissue. *J Clin Invest.* 1995;95:2111–2119.
31. Hotamisligil GS, Arner P, Caro JF, et al. Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. *J Clin Invest.* 1995;95:2409–2415.

AJH 2005; 18:457–463

Relation of Genetic Predisposition and Insulin Resistance to Left Ventricular Hypertrophy in Hypertension

Yuji Shigematsu, Yuji Hara, Tomoaki Ohtsuka, Akiyoshi Ohgimoto, Katsuji Inoue, and Jitsuo Higaki

Background: The aim of the study was to determine whether genetic predisposition to hypertension and insulin resistance are related to left ventricular (LV) hypertrophy in essential hypertension.

Methods: The study included 72 nondiabetic patients with essential hypertension and 15 normotensive control (NC) subjects. The 72 patients were divided into two groups according to genetic predisposition to hypertension. The family history (FH)(+) group included 33 patients with at least one essential hypertensive parent or sibling. The FH(−) group included 39 patients with weak genetic predisposition to hypertension. Insulin resistance was estimated using the homeostasis model assessment (HOMA). Echocardiographically determined LV mass (LVM) and relative wall thickness (RWT) were measured as markers of LV hypertrophy.

Results: The HOMA values in the FH(+) group (2.00 ± 0.89) were significantly higher than those in either the FH(−) group (1.21 ± 0.44) or NC subject group (0.91 ± 0.24). The HOMA values in the FH(−) group were significantly higher than those in NC subjects. The LVM and RWT were greatest in the FH(+) group, followed by those in the FH(−) group and NC subjects. There were no significant differences in LVM and RWT between the FH(−) group and NC subjects. By multivariate analysis, HOMA value (P = .0011), male sex (P = .0032), body mass index (P = .0061), systolic blood pressure (P = .0245), and genetic predisposition to hypertension (P = .0441) remained determinants of LVM in nondiabetic patients with essential hypertension.

Conclusions: Genetic predisposition to hypertension and the HOMA value appear to have additive impact on LV hypertrophy. This relation is independent of well-known determinants of LVM such as male sex, overweight, and high blood pressure. Am J Hypertens 2005; 18:457–463 © 2005 American Journal of Hypertension, Ltd.

Key Words: Hypertension, left ventricular hypertrophy, left ventricular geometry, genetic predisposition, insulin resistance.

Echocardiographically determined left ventricular (LV) hypertrophy is a potent independent predictor of cardiovascular morbidity and mortality in essential hypertension.[1,2] Furthermore, there is increasing evidence of a link between LV hypertrophy and hypertensive target organ damage.[3–5] Although LV mass results from the complex interaction between genetic, environmental, and lifestyle factors, both known and postulated determinants of LV mass such as elevated blood pressure (BP), male sex, obesity, and advanced age only partially explain its variability in the population.

Epidemiologic studies in twins suggest that LV hypertrophy may be influenced by genetic factors in addition to biological variables that are known to influence LV hypertrophy.[6,7] Bella et al reported the heritability of LV dimensions and mass in an American Indian population.[8] On the other hand, a number of previous studies have evaluated relations between insulin resistance and LV hypertrophy, with variably positive or negative results.[9–15] In the clinical setting, an inverse association was reported between insulin sensitivity and LV wall thickness in essential hypertension.[9] Furthermore, genetic predisposition to hypertension and insulin resistance share several physiopathologic abnormalities and are frequently associated with essential hypertension.

Accordingly, the present study was undertaken to determine whether genetic predisposition to hypertension and insulin resistance are related to the progression of LV

Received August 2, 2004. First decision October 5, 2004. Accepted October 20, 2004.

From The Second Department of Internal Medicine, Ehime University School of Medicine, Shitsukawa, Toon-city, Ehime, Japan.

Address correspondence and reprint requests to Dr. Yuji Shigematsu, The Second Department of Internal Medicine, Ehime University School of Medicine, Shitsukawa, Toon-city, Ehime 791-0295, Japan; e-mail: yujis@m.ehime-u.ac.jp

0895-7061/05/$30.00
doi:10.1016/j.amjhyper.2004.10.027

hypertrophy in nondiabetic patients with essential hypertension.

Methods

Study Population

The study population included 72 nondiabetic patients with essential hypertension (40 men and 32 women, mean age 53 ± 11 years) and 15 normotensive control (NC) subjects (10 men and five women, mean age 50 ± 13 years). All had normal findings on chemical screening battery and were nondiabetic according to the criteria of the American Diabetes Association.[16] All study patients participated after giving informed consent. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association and was approved by the Ehime University Hospital Local Ethics Committee.

A total of 72 nondiabetic patients with hypertension that had never been treated patients were divided into two groups according to the presence or absence of family history of hypertension. The family history (FH)(+) group included 33 patients (mean age, 52 ± 10 years) with at least one parent or sibling with essential hypertension before 60 years of age, as confirmed by measurement of BP values or by the ongoing use of pharmacologic antihypertensive treatment.

Blood pressure of parents or siblings was measured by sphygmomanometer two times on different days by one of the physicians. The FH(−) group included 39 patients (53 ± 11 years) who did not have parents with essential hypertension. In addition, 15 NC subjects had no parents with essential hypertension.

To exclude the presence of secondary forms of hypertension, all patients underwent a complete medical history, physical examination, and appropriate laboratory evaluation.[3]

Physical Examinations

Weight and height were measured while the subjects were fasting overnight and wearing only underwear. Body mass index (BMI) was calculated as weight (kg) divided by height $(m)^2$. Blood pressure was measured in triplicate by a single physician who was expert in the evaluation of hypertension, with an appropriate arm cuff and a mercury sphygmomanometer with the subject in sitting position after 5 min rest. The arithmetic mean of the last two measurements was calculated. Korotkoff phase V was taken for diastolic BP. Hypertension was defined as systolic BP (SBP) ≥140 mm Hg or diastolic BP (DBP) ≥ 90 mm Hg.[17]

Biochemical Investigations

In the morning after an overnight fast, venous blood was sampled for the measurement of plasma concentrations of glucose and insulin, and serum concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG). Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula: LDL-C = TC − HDL-C − TG/5. Plasma glucose was immediately determined by the glucose oxidase method. Plasma insulin was determined in duplicate by a highly specific and sensitive immunoradiometric assay (Abbott Japan; intra-assay coefficient of variation (CV) 1.6%, interassay CV 2.2%). Serum concentrations of TC, HDL-C, and TG were assessed by standard enzymatic methods.

Insulin resistance was assessed from fasting immunoreactive insulin (FIRI) and fasting plasma glucose (FPG) and the previously validated homeostasis model assessment (HOMA),[18] as follows: HOMA value = FIRI (μU/mL) × FPG (mg/dL)/405.

Echocardiographic Measurements

Two-dimensional guided M-mode echocardiography was performed by standard methods as previously outlined,[3] using an SSD-870 or SSD-5500 echocardiograph with a 3.5-MHz transducer (Aloka Inc., Tokyo, Japan). Echocardiographic examination was performed and interpreted by the same cardiologist, who was unaware of the patient's family history of hypertension and other details. The LV internal dimension (LVID), interventricular septal thickness (IVST), and posterior wall thickness (PWT) were measured at end-diastole and end-systole according to the American Society of Echocardiography guidelines,[19] and were used for all purposes except determination of LV mass. The LV mass (LVM) was calculated at end-diastole using Penn convention.[20] The LV mass/height, LV mass/body surface area (BSA), and LV mass/$height^{2.7}$ were calculated as indexed LV mass. Relative wall thickness (RWT) was also measured as follows: RWT = 2 × (PWTd/LVIDd), where d is the end-diastole.

Aortic annular cross-sectional area (in square centimeters) was calculated from the measured aortic annulus and multiplied by the aortic time–velocity integral in centimeters to yield Doppler stroke volume (SV).[21] The ratio of SV to pulse pressure was used as an indirect measure of aortic compliance.[22]

Statistical Analysis

All values are expressed as mean ± SD. The Pearson χ^2 statistic was used to analyze categorical variables. One-way analysis of variance was used to evaluate difference among groups, with the Scheffé correction for multiple comparisons. Correlation coefficients were calculated according to the Pearson method. A multivariate analysis using multiple stepwise linear regression techniques was performed to select appropriate independent variables producing the highest standardized coefficient with LVM or RWT in patients with hypertension. A forward entry stepping algorithm was used with the entry criteria probability

of $F = 0.05$. In all analyses, values of $P < .05$ were considered to be statistically significant.

Results

Demographic and Clinical Characteristics

Demographic and clinical characteristics of the three groups are shown in Table 1. There were no significant differences in age, sex distribution, body surface area, BMI, and heart rate among the three groups. Office SBP and DBP were significantly higher in hypertensive groups than in the NC subjects. However, there was no significant difference in office BP between the FH(+) group and the FH(−) group. Similarly, pulse pressure in both hypertensive groups was higher than in the NC subjects.

Biochemical Characteristics

Biochemical characteristics of the three groups are shown in Table 2. The FPG in the FH(+) group was significantly higher than that in the NC subjects. However, there was no significant difference in FPG between the FH(+) and FH(−) group. The FIRI in the FH(+) and FH(−) groups was significantly higher than that in NC subjects. In addition, the FIRI in the FH(+) group was significantly higher than in the FH(−) group. There were no significant differences in LDL-C, HDL-C, and TG among the three groups.

Echocardiographic Characteristics

Echocardiographic characteristics are shown in Table 3. The LVM, indexed LVM (LVM/height, LVM/BSA, and LVM/height$^{2.7}$) and RWT were largest in the FH(+) group, followed by those in the FH(−) group and NC subjects. There were no significant differences in LVM, LVM/BSA, and RWT between the FH(−) and NC subjects. In addition, there was no significant difference in Doppler SV among the three groups. However, the SV/PP ratio was lower in both hypertensive groups than in the NC subjects. There was no significant difference in SV/PP ratio between the FH(+) and FH(−) groups. The SV/PP ratio showed a significant negative correlation ($r = -0.390$) with the HOMA values in hypertensive patients.

Relationship Between LV Remodeling and Genetic Factors or Insulin Resistance

Figure 1 shows the HOMA values in NC subjects, the FH(+) group, and the FH(−) group. The HOMA values in the FH(+) group (2.00 ± 0.89) were significantly higher than those in the FH(−) group (1.21 ± 0.44) and NC subjects (0.91 ± 0.24). There was no significant difference in the HOMA values between the FH(−) and NC subjects.

As indicated in Fig. 2, the HOMA values showed a significant correlation with either LVM in hypertensive patients. Increasing HOMA values were related to increasing LVM in male hypertensive patients ($r = 0.587$, $P < .0001$) but not in female hypertensive patients ($r = 0.026$,

Table 1. Demographic and clinical characteristics in study subjects

	n	Age (y)	M/F	BSA (m^2)	BMI (kg/m^2)	HR (beats/min)	SBP (mm Hg)	DBP (mm Hg)	PP (mm Hg)
NC subjects	15	50 ± 13	10/5	1.55 ± 0.10	23.3 ± 2.6	63 ± 5	129 ± 5	80 ± 6	55 ± 5
Hypertension									
FH(+) group	33	52 ± 10	22/11	1.65 ± 0.15	23.6 ± 3.0	70 ± 10	166 ± 16	90 ± 13	77 ± 13
FH(−) group	39	53 ± 11	18/21	1.57 ± 0.15	24.7 ± 3.3	69 ± 11	160 ± 16	87 ± 10	76 ± 16
P value									
NC *v* FH(+)		NS	NS	NS	NS	NS	<.0001	.0026	<.0001
NC *v* FH(−)		NS	NS	NS	NS	NS	<.0001	.0263	<.0001
FH(+) *v* FH(−)		NS	NS	NS	NS	NS	NS	NS	NS

BMI = body mass index; DBP = diastolic blood pressure; F = female; FH = familial history; HR = heart rate; M = male; NC = normotensive control; NS = not significant; PP = pulse pressure; SBP = systolic blood pressure.
Data are presented as mean value ± SD.

Table 2. Biochemical characteristics in study subjects

	n	FPG (mg/mL)	FIRI (μU/mL)	LDL-C (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)
NC subjects	15	87.5 ± 9.2	4.22 ± 1.01	120 ± 16	45 ± 14	132 ± 43
Hypertension						
FH(+) group	33	99.4 ± 14.8	8.10 ± 3.03	123 ± 34	43 ± 14	128 ± 55
FH(−) group	39	92.7 ± 8.8	5.26 ± 1.84	137 ± 34	47 ± 13	135 ± 64
P value						
NC *v* FH(+)		.0059	<.0001	NS	NS	NS
NC *v* FH(−)		NS	NS	NS	NS	NS
FH(+) *v* FH(−)		NS	<.0001	NS	NS	NS

FIRI = fasting immunoreactive insulin; FPG = fasting plasma glucose; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglyceride; other abbreviations as in Table 1.
Data are presented as mean value ± SD.

not significant). Furthermore, FPG was significantly related to LVM ($r = 0.542$, $P < .0001$) or RWT ($r = 0.574$, $P < .0001$), respectively. The SV/PP ratio showed a weak but significant negative correlation with RWT ($r = -0.362$, $P = .0097$) in hypertensive patients.

Table 4 shows the results of the multivariate analysis. The HOMA value was the strongest contributor to the resultant model, with smaller and approximately equal contributions from BMI and male sex and lesser contributions from genetic predisposition of hypertension and systolic BP; age did not bear a significant relation to LVM. Independent determinants of RWT were the HOMA value and age.

Discussion

Because LV hypertrophy is strongly and independently associated with cardiovascular morbidity and mortality,[1,2] it would be helpful to be able to identify hypertensive patients who are likely to develop LV hypertrophy. We found that hypertensive patients with genetic predisposition to hypertension and insulin resistance had LV hypertrophy, including significant increases in LVM, indexed LVM, and RWT. In a multivariate analysis, HOMA value, male sex, BMI, SBP, and genetic predisposition to hypertension were independently associated with LVM. The HOMA value and age were also independently associated with RWT. Thus, we suggest that a genetic predisposition to hypertension and the HOMA value appear to have additive impacts on LV hypertrophy in patients with essential hypertension and normal glucose tolerance. This relation is independent of known and postulated determinants of LVM such as male sex, overweight, and high BP.

There is growing evidence that LV hypertrophy is influenced by genetic factors.[6–8,23] On the other hand, a number of previous studies have evaluated the relations between LV hypertrophy and insulin resistance.[9–11,13–15] Although genetic predisposition to hypertension and insulin resistance share several physiopathologic abnormalities and are frequently associated, to our knowledge, no study has looked at whether genetic factors and insulin resistance are related to the progression of LV remodeling in essential hypertension. We observed a significant relationship between LVM and genetic predisposition to hypertension. Our findings agree with previous studies[6–8,23] in which genetic factors accounted for a small but discernible proportion of the overall variance in LVM.

It is widely acknowledged that peripheral hyperinsulinemia in patients with hypertension is a marker of insulin resistance.[9,24] Diminished insulin sensitivity with regard to glucose use causes a substantial increase in insulin production in an attempt to maintain normal glucose use, making it possible that cardiovascular trophic effects and other actions of insulin could be exaggerated. The HOMA value was calculated to obtain a better quantitative estimate of insulin resistance.[25] In the present study, we showed an independent association between echocardiographically determined LVM, indexed LVM and RWT, and HOMA value in patients with hypertension, thereby confirming previous positive reports. Interestingly, the strength of association with the HOMA value, as reflected by the magnitude of standardized coefficient, increased in RWT compared with LVM. These findings agree with a previous study[13] in which several components of insulin resistance syndrome were found to be related to thick LV walls and concentric remodeling but less to LVM in elderly men.

Verdecchia et al[10] reported that insulin and insulin growth factor–1 (IGF-1) were powerful independent determinants of LVM in nondiabetic patients with hypertension. The direct effect of insulin on cardiac myocyte growth could be mediated, at least in part, by IGF-1 receptors.[26] Unfortunately, we could not determine IGF-1 binding protein in the present study. However, because fasting insulin levels were positively correlated with LVM and RWT, our data suggest that insulin is a powerful determinant of cardiac myocyte in individuals with untreated essential hypertension and normal glucose tolerance. In addition, hypertensive patients with glucose intolerance have more severe LV hypertrophy and LV diastolic dysfunction than those with normal glucose tolerance.[11,14,27]

Table 3. Echocardiographic characteristics in study subjects

	n	LVM (g)	LVM/height (g/m)	LVM/BSA (g/m^2)	LVM/height$^{2.7}$ ($g/m^{2.7}$)	RWT	Doppler SV (mL)	SV/PP ratio (mL/mm Hg)
NC subjects	15	135 ± 20	79 ± 11	87 ± 11	35 ± 5	0.32 ± 0.03	88.3 ± 13.2	1.63 ± 0.28
Hypertension								
FH(+) group	33	207 ± 55	128 ± 30	126 ± 28	57 ± 12	0.42 ± 0.09	82.7 ± 9.3	1.11 ± 0.25
FH(−) group	39	167 ± 34	106 ± 20	106 ± 19	50 ± 10	0.39 ± 0.07	85.6 ± 12.8	1.15 ± 0.24
P value								
NC *v* FH(+)		<.0001	<.0001	<.0001	<.0001	.0039	NS	<.0001
NC *v* FH(−)		NS	.0316	NS	.0075	NS	NS	<.0001
FH(+) *v* FH(−)		.0007	.0015	.0017	.0151	NS	NS	NS

BSA = body surface area; FH = familial history; LVM = left ventricular mass; NC = normotensive control; NS = not significant; SV = stroke volume; RWT = relative wall thickness; other abbreviations as in Table 1.

Data are presented as the mean value ± SD.

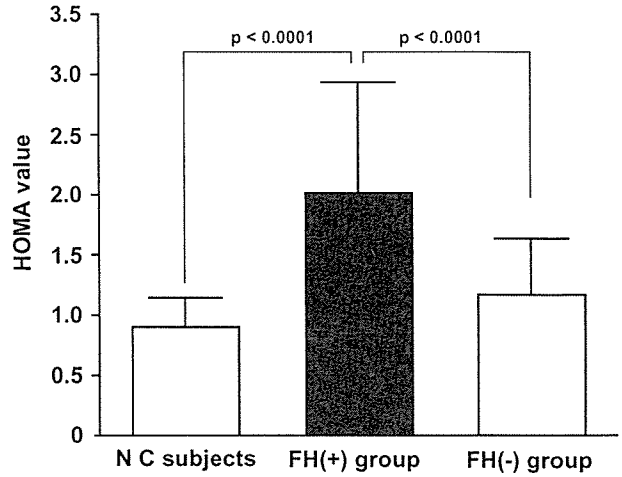


FIG. 1 Comparison of homeostasis model assessment (HOMA) values in normotensive control (NC) subjects and hypertensive patients with family history [FH(+)] and with weak family history [FH(−)]. **Column height** represents mean; **bars** indicate 95% confidence intervals.

In a recent investigation, high HOMA value was related to LVM in women alone, but this relation was largely accounted for by obesity.[15] In the present study, high HOMA values were related to LVM in male hypertensive subjects but not in female hypertensive subjects. Furthermore, this relationship was not accounted for by overweight. The absence of an association between HOMA value and LVM in female hypertensive subjects in our study might be due to the small sample size. Alternatively, it could indicate that insulin affects LV geometry differently in men and women in these Japanese hypertensive patients. When demographic variables (age, BMI, and sex distribution), SBP, genetic predisposition to hypertension, and HOMA value were considered together, the strongest

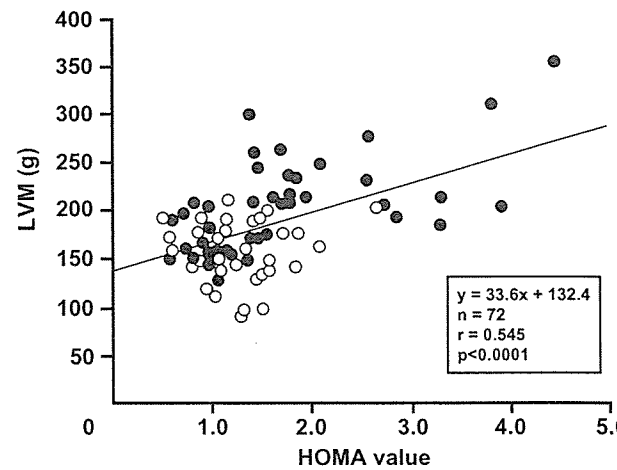


FIG. 2 Relationship between the homeostasis model assessment (HOMA) value and echocardiographically determined left ventricular mass (LVM) in female patients (**open circles**) and male patients (**closed circles**) with essential hypertension. A statistically significant positive relation was found between the HOMA value and LVM.

Table 4. Multivariate analysis of factors relevant to left ventricular mass (LVM) and relative wall thickness (RWT) in patients with hypertension

	LVM		RWT	
Variable	**Standardized coefficient**	***P* value**	**Standardized coefficient**	***P* value**
Age	−0.065	.3028	0.241	.0207
Sex	−0.284	.0032	−0.149	.1498
Body mass index	0.258	.0061	0.084	.4054
Systolic blood pressure	0.217	.0245	0.195	.0631
Genetic predisposition to hypertension	0.214	.0441	−0.138	.2416
HOMA value	0.341	.0011	0.549	<.0001
	Adjusted R^2 = 0.489, P < .0001		Adjusted R^2 = 0.333, P < .0001	

HOMA = homeostasis model assessment.

determinant of LVM was the HOMA value (positive). The contribution of male sex to the predictive model for LVM approximately equaled that of BMI. Genetic predisposition to hypertension and SBP added weakly to the multivariate model, but age did not.

In the present study, coronary risk factors such as BP, pulse pressure, heart rate, lipid profile, and vascular compliance of the large arteries as indicated by the SV/PP ratio were not significantly related to genetic predisposition to hypertension. In contrast, the SV/PP ratio was significantly related to the HOMA value in patients with essential hypertension. The independent impact of arterial status on outcome was recently found in two follow-up studies of patients with hypertension[28] or with both diabetes and glucose intolerance.[29] Together with the present results regarding hypertension and insulin resistance, a strong relationship between LV hypertrophy and cardiovascular morbidity and mortality appears to be mediated through insulin resistance.

In conclusion, there is increasing evidence of a link between insulin and cardiovascular risk,[30] although the independent role of insulin is still undetermined. The present study suggests that genetic predisposition to hypertension and the HOMA value appear to have additive impacts on LV hypertrophy. This relation is independent of well-known and postulated determinants of LVM such as male sex, overweight, and high BP.

References

1. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli MP: Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med 1990;322:1561–1566.
2. Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH: Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. Ann Intern Med 1991;114:345–352.
3. Shigematsu Y, Hamada M, Mukai M, Matsuoka H, Sumimoto T, Hiwada K: Clinical evidence for an association between left ventricular geometric adaptation and extracardiac target organ damage in essential hypertension. J Hypertens 1995;13:155–160.
4. Shigematsu Y, Hamada M, Okayama H, Hara Y, Hayashi Y, Kodama K, Kohara K, Hiwada K: Left ventricular hypertrophy precedes other target-organ damage in primary aldosteronism. Hypertension 1997;29:723–727.
5. Shigematsu Y, Hamada M, Ohtsuka T, Hashida H, Ikeda S, Kuwahara T, Kuwahara T, Hara Y, Kodama Y, Hiwada K: Left ventricular geometry as an independent predictor for extracardiac target-organ damage in essential hypertension. Am J Hypertens 1998;11:1171–1177.
6. Adams T, Yanowitz F, Fisher AG, Ridges JD, Nelson AG, Hagan AD, Williams RR, Hunt SC: Heritability of cardiac size: an echocardiographic study of monozygotic and dizygotic twins. Circulation 1985;71:39–44.
7. Harshfield GA, Grim C, Hwang C, Savage D, Anderson S: Genetic and environmental influences on echocardiographically determined left ventricular mass in black twins. Am J Hypertens 1990;3:538–543.
8. Bella JN, MacCluer JW, Roman MJ, Almasy L, North KE, Best LG, Lee ET, Fabsitz RR, Howard BV, Devereux RB: Heritability of left ventricular dimensions and mass in American Indians: the Strong Heart Study. J Hypertens 2004;22:281–286.
9. Lind L, Andersson PE, Andren B, Hanni A, Lithell HO: Left ventricular hypertrophy in hypertension is associated with the insulin resistance metabolic syndrome. J Hypertens 1995;13:433–438.
10. Verdecchia P, Reboldi G, Schillaci G, Borgioni C, Ciucci A, Telera MP, Santeusanio F, Porcellati C, Brunetti P: Circulating insulin and insulin insulin-like growth factor-1 are independent determinants of left ventricular mass and geometry in essential hypertension. Circulation 1999;100:1802–1807.
11. Galvan AQ, Galetta F, Natali A, Muscelli E, Sironi AM, Cini G, Camastra S, Ferrannini E: Insulin resistance and hyperinsulinemia: no independent relation to left ventricular mass in humans. Circulation 2000;102:2233–2238.
12. Sundstrom J, Lind L, Nystrom N, Zethelius B, Andren B, Hales CN, Lithell HO: Left ventricular concentric remodeling rather than left ventricular hypertrophy is related to the insulin resistance syndrome in elderly men. Circulation 2000;101:2595–2600.
13. Palmieri V, Bella JN, Arnett DK, Liu JE, Oberman A, Schuck MY, Kitzman DW, Hopkins PN, Morgan D, Rao DC, Devereux RB: Effect of type 2 diabetes mellitus on left ventricular geometry and systolic function in hypertensive subjects: Hypertension Genetic Epidemiology Network (HyperGEN) study. Circulation 2001;103:102–107.
14. Devereux RB, de Simone G, Palmieri V, Oberman A, Hopkins P, Kitzman DW, Rao DC, Arnett DK: Relation of insulin to left ventricular geometry and function in African American and white hypertensive adults: the HyperGEN study. Am J Hypertens 2002;15:1029–1035.

15. Rutter MK, Parise H, Benjamin EJ, Levy D, Larson MG, Meigs JB, Nesto RW, Wilson PWF, Vasan RS: Impact of glucose intolerance and insulin resistance on cardiac structure and function: sex-related differences in the Framingham Heart Study. Circulation 2003;107: 448–454.
16. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–1197.
17. National High Blood Pressure Education Program: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute: The sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Arch Intern Med 1997;157:2413–2446.
18. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 1985;28:412–419.
19. Sahn DJ, DeMaria A, Kisslo J, Weyman A, The Committee on M-Mode Standardization of the American Society of Echocardiography: Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58:1072–1083.
20. Devereux RB, Reichek N: Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. Circulation 1977;55:613–618.
21. Dubin J, Wallerson DC, Cody RJ, Devereux RB: Comparative accuracy of Doppler echocardiographic methods for clinical stroke volume determinations. Am Heart J 1990;120:116–123.
22. Ferguson JJ, Julius S, Randall OS: Stroke volume–pulse pressure relationships in borderline hypertension: a possible indicator of decreased arterial compliance. J Hypertens 1984;2(Suppl 3):397–399.
23. Post WS, Larson MG, Myers LR, Galderisi M, Levy D: Heritability of left ventricular mass: the Framingham Heart Study. Hypertension 1997;30:1025–1028.
24. Denker PS, Pollock VE: Fasting serum insulin levels in essential hypertension: a meta-analysis. Arch Intern Med 1992;152:1649–1651.
25. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, Alberiche M, Bonadonna RC, Muggeo M: Prevalence of insulin resistance in metabolic disorder: the Bruneck Study. Diabetes 1998; 47:1643–1649.
26. Strauss DS: Growth-stimulatory actions of insulin in vitro and in vivo. Endocr Rev 1984;5:356–367.
27. Hara-Nakamura N, Kohara K, Sumimoto T, Lin M, Hiwada K: Glucose intolerance exaggerates left ventricular hypertrophy and dysfunction in essential hypertension. Am J Hypertens 1994;7: 1110–1114.
28. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A: Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001;37:1236–1241.
29. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG: Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 2002;106:2085–2090.
30. Ruige JB, Assendelft WJJ, Dekker JM, Kostense PJ, Heine RJ, Bouter LM: Insulin and risk of cardiovascular disease: a meta-analysis. Circulation 1998;97:996–1001.

Original Article

Sex-Related Differences in Relations of Uric Acid to Left Ventricular Hypertrophy and Remodeling in Japanese Hypertensive Patients

Akira KURATA, Yuji SHIGEMATSU, and Jitsuo HIGAKI

Both hyperuricemia and echocardiographically determined left ventricular (LV) mass have a well-determined association with cardiovascular morbidity and mortality. However, whether or not there is a sex difference in the association of serum uric acid level with LV mass has never been systematically explored. We examined the sex-specific relation of serum uric acid level and echocardiographic indexes of LV structure in never-treated patients with essential hypertension. We enrolled 160 never-treated hypertensive patients (89 men and 71 women) to assess the possible relationship between LV mass and serum uric acid levels. LV measurements were performed according to the recommendations of the American Society of Echocardiography and the Penn Convention. LV mass was indexed by height, body surface area and height raised to the 2.7th power. A positive significant correlation between LV geometry (LV mass, indexed LV mass and relative wall thickness) and serum uric acid level was found in male hypertensive patients but not in female hypertensive patients. Independent determinants of serum uric acid levels in male hypertensive patients were LV mass and serum creatinine levels. In addition, male hypertensive patients with concentric hypertrophy showed the highest serum uric acid levels. In comparison, independent determinants of serum uric acid levels in female hypertensive patients were age and serum creatinine levels. In conclusion, these findings indicate a sex difference in the association of uric acid with LV geometry in Japanese hypertensive patients. In addition, the finding that the highest levels of serum uric acid were observed in our male hypertensive patients with concentric hypertrophy confirmed the previous reports that these patients have the highest risk for cardiovascular morbidity and mortality. (*Hypertens Res* 2005; 28: 133–139)

Key Words: **essential hypertension, serum uric acid, left ventricular mass, concentric hypertrophy**

Introduction

Many epidemiological studies have suggested that serum uric acid is a risk factor for cardiovascular disease (*1–7*). Elevated serum uric acid levels are accompanied by obesity, dyslipidemia, hypertension and insulin resistance, and all of which are also associated with increased risk for cardiovascular disease (*8*). The Chicago Industry Heart Study (*4*), a prospective 11.5-year study of 2,400 industrial workers, found serum uric acid levels to be independently associated with increased cardiovascular morbidity and mortality, but only in women. In Japan, there is a report suggesting a significant correlation between elevated serum uric acid levels and cardiovascular risk factors in large members of men, but not women (*5*). While sex-related differences in the impact of elevated serum uric acid levels on future cardiovascular disease have been reported, the sex-related specific role of serum uric acid in relation to cardiovascular disease has been unclear.

Echocardiographically determined left ventricular (LV) hypertrophy is known to be a powerful, independent risk factor of future cardiovascular morbidity and mortality in

From the Second Department of Internal Medicine, Ehime University School of Medicine, Toon, Japan.
Address for Reprints: Yuji Shigematsu, M.D., The Second Department of Internal Medicine, Ehime University School of Medicine, Shizukawa, Toon 791–0295, Japan. E-mail: yujis@m.ehime-u.ac.jp
Recieved September 21, 2004; Accepted in revised form November 17, 2004.

Table 1. Hemodynamic and Echocardiographic Characteristics in Study Subjects

Characteristic	Normotensive control subjects (n=64)	Hypertensive patients (n=160)	p values
Age (years)	55±10	58±11	ns
Male/female	39/25	89/71	
Heart rate (beats/min)	65±9	69±9	ns
Blood pressure (mmHg)			
Systole	121±12	169±18	<0.0001
Diastole	71±10	95±14	<0.0001
Pulse pressure (mmHg)	50±11	74±18	<0.0001
LV mass/BSA (g/m^2)	82.5±19.0	116.3±29.5	<0.0001
Relative wall thickness	0.32±0.06	0.40±0.07	<0.0001
Doppler stroke volume (ml)	82.8±13.4	81.6±13.7	ns
Percent fractional shortening (%)	39.9±5.5	36.3±5.8	<0.0001

Data are presented as the mean±SD. LV, left ventricular; BSA, body surface area; ns, not significant.

patients with uncomplicated essential hypertension as well as the general population (*9–12*). Furthermore, there is increasing evidence of a link between LV hypertrophy and hypertensive target organ damage (*13–15*). An association of increased LV mass with adverse outcomes has been consistently reported in men and women, but the question of whether or not the relative strength of the relation of serum uric acid level to LV mass is similar in the two sexes has never been systematically explored. Accordingly, we examined the sex-specific relation of serum uric acid level and echocardiographic indexes of LV structure in never-treated patients with essential hypertension.

Methods

Study Population

One hundred and sixty never-treated patients (89 men and 71 women) with uncomplicated essential hypertension were enrolled in the study. Patients were excluded who had a pre-existing cardiac disease, a pre-existing medical illness, such as diabetes mellitus, or M-mode echocardiograms inadequate for clearly detecting the internal lines of the interventricular septum and LV posterior wall. Sixty-four age- and sex-matched normotensive subjects (35 men and 29 women) who had no history of hypertension and no evidence of cardiac disease served as controls. All study subjects participated in this study after giving informed consent. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association.

Physical Examinations

Weight and height were measured while the subjects were fasting overnight and wearing only underwear. Body mass index (BMI) was calculated as weight (kg) divided by [height (m)]^2. Blood pressure was measured in triplicate by a single physician who was expert in the evaluation of hypertension, with an appropriate arm cuff and a mercury sphygmomanometer after 5 min of rest in the sitting position. The arithmetic mean of the last two measurements was calculated. Korotkoff phase V was taken for diastolic blood pressure. Hypertension was defined as a blood pressure above 140/90 mmHg, according to the classification of BP for adults in the Guideline for the Management of Hypertension for General Practitioners (JSH 2000) (*16*).

Biochemical Measurements

In the morning, after an overnight fast, venous blood was sampled for the measurement of serum uric acid, serum creatinine, and serum concentrations of total cholesterol (TC), high density lipoprotein (HDL) cholesterol and triglycerides (TG). Uric acid was analyzed by the uricase-peroxidase method. Normal serum uric acid levels in our laboratory are 4.3–8.0 mg/dl for men, and 2.7–5.8 mg/dl for women. Creatinine was analyzed by Jaffe's method (normal range in our laboratory: 0.5–1.2 mg/dl). The 24-h urine collections for creatinine clearance in hypertensive patients were supervised by the nursing staff. The 24-h creatinine clearance was taken as an index of glomerular filtration rate. Measurements of serum creatinine, urinary creatinine, serum uric acid, TC, HDL cholesterol and TG were carried out using an automatic analyzer (model TBA-60S; Toshiba Inc., Tokyo, Japan).

Echocardiographic Measurements

Two-dimensionally guided M-mode echocardiography was performed by standard methods, as previously outlined (*17*) using an SSD-870 or SSD-5500 echocardiograph with a 3.5 MHz transducer (Aloka Inc., Tokyo, Japan). LV internal dimension (LVID) and interventricular septal thickness (IVST) and posterior wall thickness (PWT) were measured at end-diastole and end-systole, according to the American

Table 2. Sex-Related Differences in Hemodynamic and Echocardiographic Characteristics in Hypertensive Patients

Characteristic	Male hypertensive patients (n=89)	Female hypertensive patients (n=71)	p values
Age (years)	57±11	60±11	ns
Heart rate (beats/min)	70±9	68±8	ns
Blood pressure (mmHg)			
Systole	168±18	169±17	ns
Diastole	97±16	92±12	ns
Pulse pressure (mmHg)	73±19	77±15	ns
LV mass/ BSA (g/m^2)	120.5±30.6	111.2±27.4	0.0459
Relative wall thickness	0.41±0.07	0.39±0.07	ns
Doppler stroke volume (ml)	81.5±14.1	80.6±12.6	ns
Percent fractional shortening (%)	35.0±5.9	37.9±5.4	0.0014

Data are presented as the mean±SD. LV, left ventricular; BSA, body surface area; ns, not significant.

Society of Echocardiography guidelines (*18*), and used for all purposes except determination of LV mass. LV mass was calculated at end-diastole by using the Penn convention (*19*). LV mass/height, LV mass/body surface area (BSA) and LV mass/ height$^{2.7}$ were calculated as indexed LV mass. Relative wall thickness (RWT) was calculated as 2×(PWTd/LVIDd), where d is end-diastole. Percent fractional shortening (FS) was calculated as (LVIDd − LVIDs)/LVIDd × 100 and was used as an indicator of LV systolic function, where d and s are end-diastole and end-systole, respectively. Aortic annular cross-sectional area (in cm^2) was calculated from the measured aortic annulus and multiplied by the aortic time-velocity integral in cm to yield Doppler stroke volume (SV) (*20*).

Subgroups Analysis

On the basis of the relationship between RWT and LV mass/ BSA, 89 male and 71 female hypertensive patients were then divided into 4 different groups, respectively. The partition values of 0.44 for RWT and 108 g/m^2 (male) or 104 g/m^2 (female) for LV mass/BSA, the mean+2SD value of normotensive control subjects, were used. The groups consisted of male hypertensive patients with normal RWT and LV mass/ BSA (normal geometry; n=31, 35%); patients with concentric remodeling (n=5, 6%); patients with concentric hypertrophy (n=22, 24%); and patients with eccentric hypertrophy (n=31, 35%). Furthermore, the groups consisted of female hypertensive patients with normal RWT and LV mass/BSA (normal geometry; n=27, 38%); patients with concentric remodeling (n=5, 7%); patients with concentric hypertrophy (n=12, 17%); and patients with eccentric hypertrophy (n=27, 38%).

Statistical Analysis

All values are expressed as the mean±SD. Two tailed unpaired Student's t-test was used to compare study response variables between categories. Correlation coefficients were calculated according to Pearson's method. A multiple regression analysis was also performed to select appropriate independent variables producing the highest partial correlation with serum uric acid level in patients with hypertension. Probability values <0.05 were considered statistically significant in all analyses.

Results

Hemodynamic and Echocardiographic Characteristics

Office systolic blood pressure, diastolic blood pressure or pulse pressure was significantly higher in hypertensive patients than that in normotensive control subjects. LV mass/ BSA and RWT were also larger in hypertensive patients than those in normotensive control subjects. Although Doppler stroke volume did not differ significantly, percent fractional shortening in hypertensive patients was lower than that in normotensive control subjects (Table 1).

Sex-Related Differences in Hemodynamic and Echocardiographic Characteristics

There were no significant differences in age, heart rate, systolic blood pressure, diastolic blood pressure and pulse pressure between male and female hypertensive patients. Although LV mass/BSA in male hypertensive patients was larger than that in female hypertensive patients, RWT and Doppler stroke volume did not differ significantly. Furthermore, percent fractional shortening in female hypertensive patients was higher than that in male hypertensive patients (Table 2).

Sex-Related Differences in Biochemical Characteristics

Although there was no significant difference in the 24-h crea

Table 3. Sex-Related Differences in Biochemical Characteristics in Hypertensive Patients

Characteristic	Male hypertensive patients (n=89)	Female hypertensive patients (n=71)	p values
Serum uric acid (mg/dl)	6.75±1.39	5.46±1.07	<0.0001
Serum creatinine (mg/dl)	0.99±0.31	0.75±0.20	<0.0001
Creatinine clearance (ml/min)	84.5±28.1	91.9±24.2	ns
Body mass index (g/m^2)	24.1±2.3	24.5±3.9	ns
Total cholesterol (mg/dl)	199±39	217±40	0.0055
HDL cholesterol (mg/dl)	40±12	47±14	0.0031
Triglyceride (mg/dl)	139±61	118±36	ns

Data are presented as the mean±SD. HDL, high density lipoprotein; ns, not significant.

Table 4. Simple Correlation of Serum Uric Acid Level with Age, Left Ventricular Geometry and Function in Male and Female Patients with Hypertension

Variable	Serum uric acid level			
	Male HT (n=89)		Female HT (n=71)	
	r values	p values	r values	p values
Age	0.037	0.7310	0.307	0.0092
LV mass	0.399	0.0001	0.134	0.2646
LV mass/height	0.353	0.0007	0.150	0.2115
LV mass/BSA	0.371	0.0004	0.151	0.2086
LV mass/height$^{2.7}$	0.355	0.0006	0.174	0.1458
RWT	0.324	0.0019	0.018	0.8848
Percent FS	0.038	0.7268	0.080	0.5061

HT, hypertension; LV, left ventricular; BSA, body surface area; RWT, relative wall thickness; FS, fractional shortening.

tinine clearance between male and female hypertensive patients, serum uric acid and serum creatinine levels in male hypertensive patients were significantly higher than those in female hypertensive patients. Both total and HDL cholesterol levels in female hypertensive patients were significantly higher than those in male hypertensive patients. There were no significant differences in BMI and TG levels between the male and female hypertensive groups (Table 3).

Correlations between Serum Uric Acid Levels and LV Geometry or Serum Lipid Levels

As shown in Table 4, serum uric acid levels were significantly related to LV mass, LV mass/height, LV mass/BSA, LV mass/height$^{2.7}$ and RWT in male hypertensive patients. However, serum uric acid levels were not significantly related to LV geometry (LV mass, indexed LV mass and RWT) in female hypertensive patients. Serum uric acid levels were not significantly related to percent FS in either male or female hypertensive patients. Furthermore, serum uric acid levels were not significantly related to BMI, TC, HDL cholesterol or TG levels in either male or female hypertensive patients. Table 5 shows the results of multiple regression analysis. Independent determinants of serum uric acid levels in male hypertensive patients were LV mass and serum creatinine levels. In contrast, independent determinants of serum uric acid levels in female hypertensive patients were age and serum creatinine levels. In addition, Fig. 1A shows the comparison of serum uric acid levels in male hypertensive patients with normal geometry (6.1±1.1 mg/dl), concentric remodeling (6.9±1.6 mg/dl), concentric hypertrophy (7.5±1.2 mg/dl) and eccentric hypertrophy (6.8±1.5 mg/dl). Male hypertensive patients with concentric hypertrophy showed the highest serum uric acid levels. However, there were no significant correlations between serum uric acid levels and LV geometry in female hypertensive patients. Figure 1B shows the comparison of serum uric acid levels in female hypertensive patients with normal geometry (5.1±0.9 mg/dl), concentric remodeling (5.7 ± 1.3 mg/dl), concentric hypertrophy (5.2±1.2 mg/dl) and eccentric hypertrophy (5.8±1.1 mg/dl).

Discussion

In this cross-sectional study, serum uric acid levels correlated positively with LV geometry (LV mass, indexed LV mass and RWT) in male hypertensive patients, but not in female hypertensive patients. In comparison, serum uric acid levels correlated positively with age in female hypertensive patients, but not in male hypertensive patients. These findings indicate a sex difference in the association of uric acid with LV geome-

Table 5. Multiple Regression Analysis of Factors Relevant to Serum Uric Acid Level in Male and Female Patients with Hypertension

Variable	Serum uric acid level					
	Male HT (n=89)			Female HT (n=71)		
	β	t values	p values	β	t values	p values
Age	−0.075	−0.729	0.4682	0.422	3.109	0.0028
Body mass index	0.012	0.123	0.9027	0.184	1.564	0.1226
LV mass	0.359	3.567	0.0006	0.059	0.487	0.6279
Serum creatinine	0.316	3.181	0.0021	0.306	2.478	0.0159
Systolic BP	−0.007	−0.065	0.9481	−0.017	−0.133	0.8947
Diastolic BP	−0.132	−1.190	0.2373	0.029	0.189	0.8507
	Multiple R^2 =0.213, p=0.0003			Multiple R^2 =0.258, p=0.0151		

HT, hypertension; LV, left ventricular; BP, blood pressure.

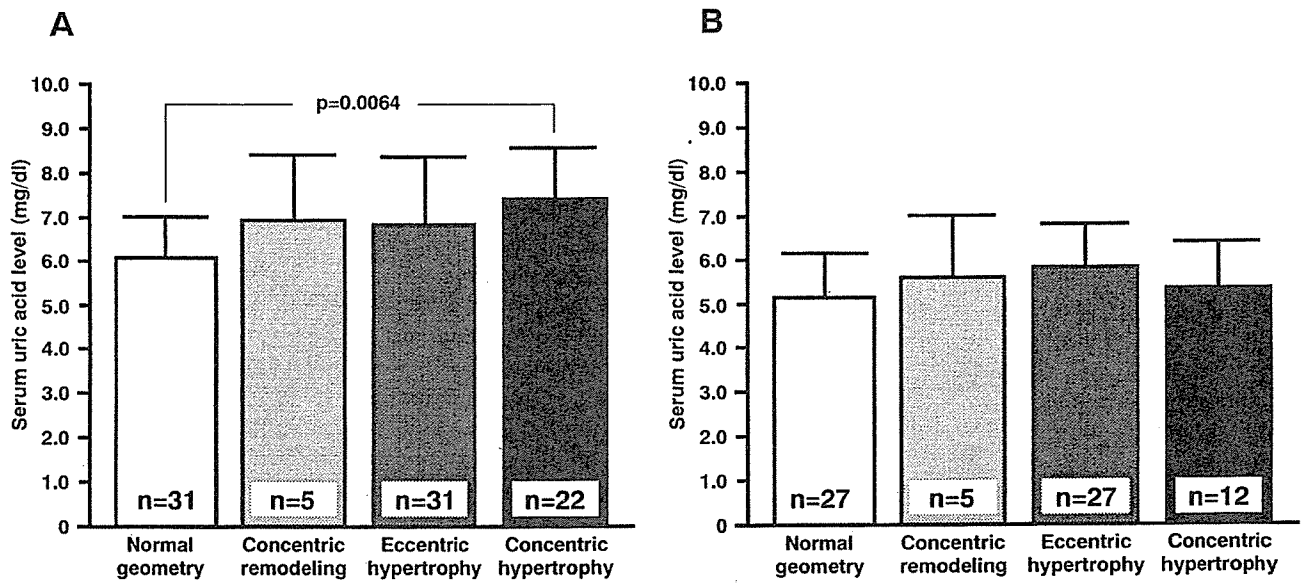


Fig. 1. *Comparison of the serum uric acid levels in male (A) and female (B) hypertensive patients with normal geometry, concentric remodeling, concentric hypertrophy and eccentric hypertrophy. Column height represents the mean; bars indicate 95% confidence intervals.*

try in Japanese hypertensive patients.

At least two prior investigations that performed sex-specific analyses reported an association of hyperuricemia with increased cardiovascular morbidity and mortality in women but not men (*4, 21*). In contrast, one previous report has suggested a significant correlation between hyperuricemia and cardiovascular risk factors in large members of men, but not women in Japan (*5*). Furthermore, it is well recognized that increased LV mass is an independent predictor for cardiovascular morbidity and mortality in both men and women. Our results provide the first evidence that serum uric acid levels in male hypertensive patients are positively correlated with echocardiographic LV mass but not in female hypertensive patients. The present data also demonstrate that when we classify male hypertensive patients on the basis of their pattern of LV geometry, the highest serum uric acid levels are evident in patients with concentric hypertrophy.

Our clinical study design did not enable us to clearly state why serum uric acid levels are correlated with LV mass only in male hypertensive patients. However, our data are consistent with Japanese epidemiological evidence that hyperuricemia may be associated with increased cardiovascular events in men, but not in women (*5*). Thus, our findings add further support to the concept that serum uric acid level is a sensitive indicator of the future cardiovascular events in Japanese male patients with hypertension. However, it should be emphasized that the serum uric acid levels reported in this study were derived from fasting blood. It is well recognized that serum uric acid levels in humans have circadian rhythm. Elevated serum uric acid levels accentuate after ingestion of a

high-protein meal or alcohol intake, but not after a low-protein meal. Thus, fasting levels may not adequately reflect the effect of uric acid on coronary circulation.

Different indexes of LV mass have been proposed for normalization of mass for body size (*9*, *22*, *23*). The optimal index, if a generalizable one exists, is still undefined, and the use of differing indexes in the literature can cause confusion. In the present study, the use of 3 different indexes of LV mass, which were normalized LV mass for body size resulted in no significant differences in the major findings.

Age was an independent predictor for the elevation of serum uric acid level in female hypertensive patients. The relation between age and the occurrence of hyperuricemia differed between men and women. Hyperuricemia correlated negatively with age in men but positively with age in women (*5*, *24*, *25*). In our study, a positive correlation between serum uric acid level and age was also recognized in female hypertensive patients. Interactions of sex hormones have been suggested as a possible cause of the difference in correlation between serum uric acid level and age in men and women (*4*). Furthermore, the LV wall thickness and LV mass have been shown to significantly increase with advancing age in healthy normotensive subjects (*26*, *27*). Therefore, the absence of an association between serum uric acid level and LV mass in female hypertensive patients in the present study may have been due to the small sample size.

In the early stage of hypertension, the serum uric acid level increases as renal blood flow decreases without affecting the glomerular filtration rate (*28*). Therefore, it certainly is possible that uric acid may be an earlier and more sensitive marker of decreased renal blood flow than serum creatinine. Furthermore, there is increasing evidence that hyperuricemia is associated with a multimetabolic syndrome (*29*) in which insulin-mediated renal hemodynamic abnormalities lead to hypertensive renal damage (*30*, *31*). Facchini *et al.* (*32*) have suggested that resistance to insulin-mediated glucose uptake and/or the compensatory hyperinsulinemia associated with a multimetabolic syndrome decrease urinary uric acid clearance, with a subsequent elevation of serum uric acid level. One of the limitations in our study was the lack of information on potentially important characteristics such as fasting plasma glucose and insulin level. Unfortunately, we could not determine the impact of insulin resistance on hyperuricemia in our study.

Despite the wealth of evidence that serum uric acid level is associated with a number of metabolic abnormalities, the relationship between serum uric acid level and the severity of hypertension is not yet fully understood. In our study, multivariate analysis revealed that serum creatinine level is an independent predictor of serum uric acid level in both male and female hypertensive patients. However, in all study patients, serum creatinine levels were within normal limits. Further investigations are needed to identify whether serum creatinine is merely a marker for renal dysfunction or plays a causative role in the incidence of hyperuricemia.

An elevated serum uric acid level may reflect impaired endothelial integrity, in which the endothelial-dependent vascular relaxation produced by nitric oxide is reduced. In addition, activation of the renin-angiotensin-aldosterone system in patients with essential hypertension plays important roles in the pathogenesis of myocardial hypertrophy (*33*) and the elevation of serum uric acid level. These processes contribute to the evolution of atherosclerosis (*34*, *35*). The association between LV mass and serum uric acid level, as reported in our study, provides a potential and earlier pathophysiological link to explain the increased risk of vascular events in hypertensive patients with LV hypertrophy. Both LV hypertrophy and elevated serum uric acid in hypertensive patients may be considered indicative of preclinical cardiovascular disease that may be reversed by the effective therapeutic interventions (*36–38*). Verdecchia *et al.* reported that the decrease in LV mass brought about by antihypertensive therapy is associated with a reduced risk for subsequent events (*36*). Furthermore, the decreasing of uric acid by allopurinol in patients with diabetes mellitus and congestive heart failure has been shown to result in improved endothelial-dependent vasodilatation (*37*). Thus, the finding that hyperuricemia and LV hypertrophy may be reversed by a pharmacological treatment may suggest a new target for therapeutic intervention in essential hypertension.

Finally, a previous prospective study has demonstrated that patients with concentric hypertrophy have a higher incidence of cardiovascular events than patients with other types of LV geometry (*10*). In the present study, the finding that the highest levels of serum uric acid were observed in our male hypertensive patients with concentric hypertrophy confirms the previous reports that these patients have the highest risk for cardiovascular morbidity and mortality (*9–12*).

References

1. Gertler MM, Garn SM, Levine SA: Serum uric acid in relation to age and physique in health and in coronary heart disease. *Ann Intern Med* 1951; **34**: 1421–1431.
2. Brand FN, McGee DL, Kannel WB, Stokes J, Castelli WP: Hyperuricemia as a risk factor of coronary heart disease: the Framingham Study. *Am J Epidemiol* 1985; **121**: 11–18.
3. Abbott RD, Brand FN, Kannel WB, Castelli WP: Gout and coronary disease: the Framingham Study. *J Clin Epidemiol* 1988; **41**: 237–242.
4. Levine W, Dyer AR, Shekelle RB, Shekelle RB, Schoenberger JA, Stamler J: Serum uric acid and 11.5-year mortality of middle-aged women: findings of the Chicago Heart Association Detection Project in Industory. *J Clin Epidemiol* 1989; **42**: 257–267.
5. Nakanishi N, Tatara K, Nakamura K, Suzuki K: Risk factors for the incidence of hyperuricemia: a 6-year longitudinal study of middle-aged Japanese men. *Int J Epidemiol* 1999; **28**: 888–893.
6. Franse LV, Pahor M, DiBari M, *et al*: Serum uric acid, diuretic treatment and risk of cardiovascular events in the Systolic Hypertension in the Elderly Program (SHEP). *J Hypertens* 2000; **18**: 1149–1554.

7. Inoue T, Matsuoka M, Nagahama K, *et al*: Cardiovascular risk factors associated with pulse pressure in screened cohort in Okinawa, Japan. *Hypertens Res* 2003; **26**: 153–158.
8. Nagahama K, Iseki K, Inoue T, Touma T, Ikemiya Y, Takishita S: Hyperuicemia and cardiovascular risk factor clustering in a screened cohort in Okinawa, Japan. *Hypertens Res* 2004; **27**: 227–233.
9. Casale PN, Devereux RB, Milner M, *et al*: Value of echocardiographic measurement of left ventricular mass in predicting cardiovascular morbid events in hypertensive men. *Ann Intern Med* 1986; **105**: 173–178.
10. Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH: Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. *Ann Intern Med* 1991; **114**: 345–352.
11. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP: Left ventricular mass and incidence of coronary heart disease in an elderly cohort: the Framingham Heart Study. *Ann Intern Med* 1989; **110**: 101–107.
12. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP: Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322**: 1561–1566.
13. Shigematsu Y, Hamada M, Mukai M, Matsuoka H, Sumimoto T, Hiwada K: Clinical evidence for an association between left ventricular geometric adaptation and extracardiac target organ damage in essential hypertension. *J Hypertens* 1995; **13**: 155–160.
14. Shigematsu Y, Hamada M, Ohtsuka T, *et al*: Left ventricular geometry as an independent predictor for extracardiac target organ damage in essential hypertension. *Am J Hypertens* 1998; **11**: 1171–1177.
15. Cuspidi C, Meani S, Salerno M, *et al*: Cardiovascular target organ damage in essential hypertensive with or without reproducible nocturnal fall in blood pressure. *J Hypertens* 2004; **22**: 273–280.
16. Japanese Society of Hypertension Guideline Subcommittee for the Management of Hypertension: Guideline for the management of hypertension for general practitioners. *Hypertens Res* 2001; **24**: 613–634.
17. Shigematsu Y, Hamada M, Okayama H, *et al*: Left ventricular hypertrophy precedes other target-organ damage in primary aldosteronism. *Hypertension* 1997; **29**: 723–727.
18. Sahn DJ, DeMaria A, Kisslo J, Weyman A: The Committee on M-Mode Standardization of the American Society of Echocardiography: Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978; **58**: 1072–1083.
19. Devereux RB, Reichek N: Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation* 1977; **55**: 613–618.
20. Dubin J, Wallerson DC, Cody RJ, Devereux RB: Comparative accuracy of Doppler methods for clinical stroke volume determinations. *Am Heart J* 1990; **120**: 116–123.
21. Freedman DS, Williamson DF, Gunter EW, Byers T: Relation of serum uric acid to mortality and ischemic heart disease: the NHANES I Epidemiologic Follow-up Study. *Am J Epidemiol* 1995; **141**: 637–644.
22. de Simone G, Devereux RB, Daniels SR, Koren MJ, Meyer RA, Laragh JH: Effect of growth on variability of left ventricular mass: assessment of allometric signals in adult and children and their capacity to predict cardiovascular risk. *J Am Coll Cardiol* 1995; **25**: 1056–1062.
23. Levy D, Savage DD, Garrison RJ, Anderson D, Kannel WB, Castelli WP: Echocardiographic criteria for left ventricular hypertrophy: the Framingham Heart Study. *Am J Cardiol* 1997; **59**: 956–960.
24. Culleton BF, Larson MG, Kannel WB, Levy D: Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. *Ann Intern Med* 1999; **131**: 7–13.
25. Fang J, Alderman MH: Serum uric acid and cardiovascular motality: the NHANES I Epidemiologic Follow-up Study, 1971–1992. *JAMA* 2000; **283**: 2404–2410.
26. Gerstenblith G, Frederiksen J, Yin FC, Fortuin NJ, Lakatta EG, Weisfeldt ML: Echocardiographic assessment of a normal adult aging population. *Circulation* 1977; **56**: 273–278.
27. Pearson AC, Gudipati CV, Labovitz AJ: Effects of aging on left ventricular structure and function. *Am Heart J* 1991; **121**: 871–875.
28. Messerli FH, Frohlich ED, Dreslinski GR, Suarez DH, Aristimuno GG: Serum uric acid in essential hypertension: an indicator of renal vascular involvement. *Ann Intern Med* 1980; **93**: 817–821.
29. Grundy SM: Small LDL, atherogenic dyslipidemia, and the metabolic syndrome. *Circulation* 1997; **95**: 1–4.
30. Ward HJ: Uric acid as an independent risk factor in the treatment of hypertension. *Lancet* 1998; **352**: 670–671.
31. Johnson RJ, Kang D-H, Feig D, *et al*: Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? *Hypertension* 2003; **41**: 1183–1190.
32. Facchini F, Chen Y-DI, Hollenbeck CB, Reaven GM: Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. *JAMA* 1991; **266**: 3008–3011.
33. Iwashima Y, Horio T, Kuroda S, *et al*: Influence of plasma aldosterone on left ventricular geometry and diastolic function in treated essential hypertension. *Hypertens Res* 2002; **25**: 49–56.
34. Bagnati M, Perugini C, Cau C, Bordone R, Albano E, Bellomo G: When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: a study using uric acid. *Biochem J* 1999; **340**: 143–152.
35. Furumoto T, Saito N, Dong J, *et al*: Association of cardiovascular risk factors and endothelial dysfunction in Japanese hypertensive patients: Implications for early atherosclerosis. *Hypertens Res* 2002; **25**: 475–480.
36. Verdecchia P, Reboldi G, Schillaci G, *et al*: Circulating insulin and insulin-like growth factor-1 are independent determinants of left ventricular mass and geometry in essential hypertension. *Circulation* 1999; **100**: 1802–1807.
37. Butler R, Morris AD, Belch JJF, Hill A, Struthers AD: Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension* 2000; **35**: 746–751.
38. Høieggen A, Alderman MH, Kjeldsen SE, *et al*: The impact of serum uric acid on cardiovascular outcomes in the LIFE study. *Kidney Int* 2004; **65**: 1041–1049.

J Mol Med (2005) 83: 619–625
DOI 10.1007/s00109-005-0641-9

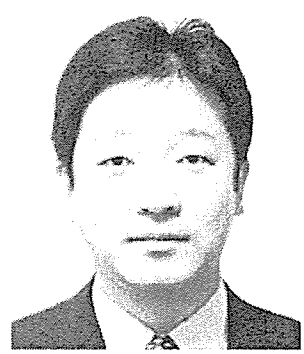
ORIGINAL ARTICLE

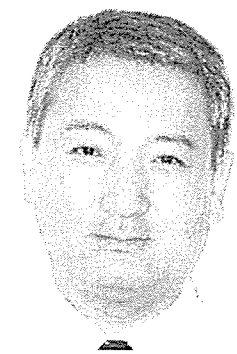
Akiyoshi Ogimoto · Yuji Shigematsu · Jun Nakura · Yuji Hara · Tomoaki Ohtsuka · Katsuhiko Kohara · Mareomi Hamada · Tetsuro Miki · Jitsuo Higaki

Endothelial nitric oxide synthase gene polymorphism (Glu298Asp) in patients with coexistent hypertrophic cardiomyopathy and coronary spastic angina

Received: 13 September 2004 / Accepted: 14 December 2004 / Published online: 19 March 2005

Abstract Coronary vasospasm appears to play a significant role in the etiology of myocardial ischemia in patients with hypertrophic cardiomyopathy (HCM). Furthermore, the management of patients with coexistent HCM and coronary spastic angina (CSA) presents a therapeutic challenge. The purpose of this study was to examine the Glu 298Asp variant of the endothelial nitric oxide synthase (*eNOS*) gene to determine whether this polymorphism was associated with susceptibility to CSA in patients with HCM. The *eNOS* gene polymorphism (Glu298Asp) was genotyped in 150 HCM patients by the TaqMan chemical method. Patients were classified into group A (n=12) if they had CSA provoked by intracoronary acetylcholine, and group B (n=138) if they did not. In group A, the frequency of Glu/Glu, Glu/Asp, and Asp/Asp genotypes was 5 (41.7%), 6 (50%), and 1 (8.3%), respectively. In group B, it was 119 (86.2%), 17 (12.3%), and 2 (1.5%), respectively. The frequency of the Asp298 variant was significantly higher in group A than in group B (P<0.001). Multivariate logistic regression analysis showed that the Asp298 variant was a significant risk factor for CSA (odds ratio 11.8; P<0.001) that was independent of age, gender, smoking status or body mass index. Significantly more drugs were used by the patients in group A than those in group B and the patients with the Asp298 variant were treated with significantly more drugs than those without it. In conclusion, the Asp298 variant of the *eNOS* gene may be associated with CSA in HCM patients. HCM patients with CSA or the Asp298 variant may need more drugs to relieve their symptoms.





Akiyoshi Ogimoto received his M.D. in 1995, and his Ph.D. in 2002 from Ehime University School of Medicine, Ehime, Japan. He is a staff physician in the 2nd Department of Internal Medicine in Ehime University School of Medicine. His research interests include the genetic risk factors for cardiovascular diseases.

Jitsuo Higaki received his M.D. in 1978, and his Ph.D. in 1985 from Osaka University School of Medicine, Osaka, Japan. He is presently Professor and Chairman of the 2nd Department of Internal Medicine (Cardiology, Pulmonology, Hypertension and Nephrology), Ehime University School of Medicine, Ehime Japan. His research interests include the molecular biology of cardiovascular diseases.

A. Ogimoto (✉) · Y. Shigematsu · Y. Hara · T. Ohtsuka · J. Higaki
The Second Department of Internal Medicine,
Ehime University School of Medicine,
Shitsukawa, Toon,
Ehime, 791-0295, Japan
e-mail: aogimoto@m.ehime-u.ac.jp
Tel.: +81-89-9605302
Fax: +81-89-9605306

J. Nakura · K. Kohara · T. Miki
Department of Geriatric Medicine,
Ehime University School of Medicine,
Ehime, Japan

M. Hamada
Department of Cardiology, Uwajima City Hospital,
Uwajima, Japan

Keywords Cardiomyopathy · Gene polymorphism · Nitric oxide · Coronary artery disease

Abbreviations HCM: Hypertrophic cardiomyopathy · CSA: Coronary spastic angina · eNOS: Endothelial nitric oxide synthase · PCR: Polymerase chain reaction

Introduction

Chest pain is a common symptom in hypertrophic cardiomyopathy (HCM). Myocardial ischemia plays an important role in the pathophysiology and natural history of HCM, and occurs even in the absence of significant epicardial coronary atherosclerosis [1]. Coronary vasospasm appears to play a significant role in the etiology of myocardial ischemia and to be associated with angina and sudden death in patients with HCM [1]. Coronary vasospasm can be induced by ergonovine maleate or acetylcholine in 9.5–39% of patients with HCM [1–3]. These induction rates are higher than in other types of heart disease, with the exception of ischemic heart disease [2]. These findings suggest that coronary vasospasm in patients with HCM may occur more frequently than has been previously recognized.

Beta-adrenergic receptor and calcium channel blocking agents are the mainstay of medical therapy for symptomatic HCM. Beta-blockers can be effective in relieving symptoms in patients with severe chest pain, dyspnea, and syncope during exertion caused by left ventricular outflow tract obstruction [4]. Patients who do not respond to beta-blockers often have symptomatic improvement if given verapamil [4]. Furthermore, some investigators administer verapamil if chest pain is the predominant symptom [5]. These findings suggest that coronary vasospasm may be important in some patients with HCM. The coexistence of HCM and CSA may affect the response to beta-blockers, which are generally contraindicated in CSA.

A recent report shows that the Glu298Asp variant in the endothelial nitric oxide synthase (*eNOS*) gene is significantly associated with CSA [6]. It would be useful to know whether patients with HCM have a genetic risk factor for CSA. However, the implication of this polymorphism with respect to HCM with coexistent CSA remains to be established. In the present study, we investigated the Glu298Asp variant of the *eNOS* gene to determine whether this polymorphism was associated with increased susceptibility to CSA in patients with HCM.

Materials and methods

Study population

The study protocols were approved by the Ethics Committee of Ehime University School of Medicine, and written and informed consent was obtained from each subject.

A series of 150 patients with HCM referred to the Department of Cardiology at the Ehime University Hospital were recruited into this study between March 2000 and January 2003. About one third of our HCM patients were referred to our hospital by local physicians. HCM was diagnosed on the basis of echocardiographic criteria defined as the presence of left ventricular hypertrophy in the absence of other causes of hypertrophy. These patients also met the definition and classification proposed by the 1995 World Health Organization/International Society and Federation of Cardiology Task Force [7]. Patients were excluded from this study if they had prior myocardial infarction or other significant heart problems such as valvular heart disease or congenital heart disease.

Two groups of patients were studied. Group A consisted of 12 HCM patients diagnosed with CSA. All 12 patients had had an episode of spontaneous angina at rest associated with ischemic ST segment changes on the 12-lead electrocardiogram (ECG) or ambulatory ECG. In addition, all group A patients lacked significant organic stenosis as documented by coronary angiography; and had coronary vasospasm associated with ischemic ST segment changes after intracoronary acetylcholine, as reported previously [8, 9]. Group B consisted of 138 HCM patients without a diagnosis of CSA. Patients were diagnosed with obstructive HCM based on the presence of a pressure gradient across the left ventricular outflow tract. Left ventricular outflow tract obstruction was defined by a pressure gradient ≥30 mmHg [4]. All previous cardiac studies in the patients were reviewed and the initial clinical status of the patients were determined on the basis of their medical records. All patients completed a standard questionnaire on their personal medical history, family history, and smoking habits. Patients that had smoked at least 1 cigarette per day for at least the previous 3 months or had stopped smoking for <1 year were classified as smokers; those that had quit smoking for at least 1 year were classified as non-smokers.

Determination of *eNOS* genotypes

Genomic DNA was extracted from peripheral blood samples with an extraction kit (Qiagen, Hilden, Germany). We determined the *eNOS* gene polymorphism by using the TaqMan (Roche Molecular Systems, Pleasanton, Calif.) polymerase chain reaction (PCR) chemistry method, as previously described [10–12]. The TaqMan probe is a fluorogenic probe that consists of an oligonucleotide labeled with both a fluorescent reporter dye and a quenched dye. The fluorescent reporter dye, such as VIC or FAM, is covalently linked to the 5′ end of the nucleotide. Each of the reporters is quenched by a minor groove binder, typically located at the 3′ end. The following primers were used for the Glu298Asp variant in the *eNOS* gene: a forward primer, 5′-GCTGCCCCTGCTGCTG-3′; a reverse primer, 5′-GGC ACCTCAAGGACCAGCT-3′; a C allele-specific probe, 5′-VIC-CCAGATGAGCCCC-MGB-3′; and an A allele-specific probe, 5′-FAM-CCAGATGATCCCC-MGB-3′. The fluorescence level of the PCR products was measured with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The investigator who assessed the genotype was blind to the clinical data of the subjects from whom the samples originated.

Echocardiography

Two-dimensional and M-mode echocardiography were performed using conventional methods. In summary, the end-diastolic left ventricular wall thickness was recorded at the

Table 1 Characteristics of the patients. Values are mean ± SD. The number of drugs shows the mean number of drug combination (beta-blockers, calcium antagonists, and class la drugs). *NYHA* New York Heart Association, *HNCM* hypertrophic non-obstructive cardiomyopathy, *HOCM* hypertrophic obstructive cardiomyopathy, *LVWT* left ventricular wall thickness, *LVED/LVES* left ventricular end-diastolic/systolic dimension, *CSA* coronary spastic angina, *n.s.* not significant

[a]Mann-Whitney *U* test
[b]Chi-square test

	HCM+CSA− (*n*=138)	HCM+CSA+ (*n*=12)	*P* value
Age (years)	62±13	60±11	n.s.[a]
Male gender (%)	107 (78%)	10 (83%)	n.s.[a]
Body mass index	24.3±3.3	24.1±3.1	n.s.[a]
NYHA class (II/III+VI)	62/13	7/3	n.s.[b]
HNCM	101 (73%)	6 (50%)	n.s.[b]
HOCM	37 (27%)	6 (50%)	n.s.[b]
Maximal LVWT	17±5	19±4	n.s.[a]
LVED	48±6	44±6	n.s.[a]
LVES	30±7	27±5	n.s.[a]
Smoking	49 (36%)	8 (67%)	0.04[b]
Beta-blockers	62 (45%)	8 (67%)	n.s.[b]
Calcium antagonists	109 (79%)	11 (92%)	n.s.[b]
Class la drugs	27 (20%)	5 (42%)	n.s.[b]
Number of drugs	1.4	2.1	<0.01[a]

mitral valve and papillary muscle level in the septal and posterior wall, as well as in the lateral and posterior left ventricular wall using short-axis two dimensional images. The maximal wall thickness was assessed from the apical four-chamber views. Left ventricular outflow tract velocities were determined using continuous wave Doppler echocardiography, and left ventricular outflow tract gradients were calculated using the modified Bernoulli equation.

Coronary angiographic study

All antianginal medications except sublingual nitroglycerin were withheld for at least 24 h before the study. Coronary angiography was performed as reported previously [8]. After neither significant atherosclerotic stenosis nor spontaneous vasospasm in the large epicardial coronary arteries had been observed on baseline angiography, the acetylcholine provocation test was performed according to a previously described method [8, 9]. Coronary vasospasm was defined as total or subtotal vasoconstriction of the epicardial coronary arteries associated with an attack of chest pain or ischemic ST segment changes.

Statistical analysis

All statistical analyses were performed on a personal computer with SPSS v10.0J for Windows (SPSS, Chicago, Ill.). Summary data were expressed as mean ± SD. Measured variables were compared between the two groups with the Mann-Whitney *U* test due to the non-normal distribution of most parameters. Categorical variables were compared by the chi-square test. Differences in the prevalence among groups and the Hardy-Weinberg equilibrium were analyzed by the chi-square method. To analyze the differences between Asp allele carriers and non-carriers, the Asp/Asp and Asp/Glu genotypes were pooled into one group. Association between polymorphisms and case/control status was tested by logistic regression analysis controlling for age, gender, body mass index and smoking. Odds ratios (ORs) were estimated with 95% confidence intervals as measures of risk. A probability value of <0.05 was considered statistically significant.

Results

Table 1 shows the clinical characteristics of the patients. No significant differences were found in age, gender, body mass index, New York Heart Association (NYHA) functional classification, type of HCM (obstructive versus non-obstructive), and echocardiographic data between the two groups. However, smokers were more frequent in the patients with CSA than those without CSA. HCM patients with CSA were treated with significantly more drugs than those without CSA. In addition, HCM patients with the Asp298 variant were treated with significantly more drugs

Table 2 Frequencies of the *eNOS* gene polymorphism. *HCM* Hypertrophic cardiomyopathy, *CSA* coronary spastic angina. The statistical significance was determined by the chi-square test

	HCM (*n*=150)	HCM+CSA− (*n*=138)	HCM+CSA+ (*n*=12)	*P* value (CSA− versus CSA+)
Asp/Asp	3 (2%)	2 (2%)	1 (8%)	
Asp/Glu	23 (15%)	17 (12%)	6 (50%)	
Glu/Glu	124 (83%)	119 (86%)	5 (42%)	0.0004
Asp/Asp+Asp/Glu	26 (17%)	19 (14%)	7 (58%)	
Glu/Glu	124 (83%)	119 (86%)	5 (42%)	<0.0001
Asp allele	29 (10%)	21 (8%)	8 (33%)	
Glu allele	271 (90%)	255 (92%)	16 (67%)	<0.0001

Table 3 Odds ratios for coronary spastic angina in patients with HCM, determined by logistic regression analysis at the 95% confidence interval. *HOCM* Hypertrophic obstructive cardiomyopathy, *HNCM* hypertrophic non-obstructive cardiomyopathy

	Univariate model		Multivariate model	
	Odds ratio	*P* value	Odds ratio	*P* value
Age	0.99 (0.95–1.0)	0.55		0.93
Male (versus female)	1.45 (0.30–7.0)	0.64		0.60
Body mass index	0.98 (0.82–1.2)	0.83		0.59
HOCM (versus HNCM)	2.7 (0.83–9.0)	0.10		0.06
Smoking	3.6 (1.0–12.7)	0.04	4.9 (1.1–22.6)	0.04
Asp allele (versus Glu/Glu)	8.8 (2.5–33.3)	<0.001	11.8 (2.9–47.6)	<0.001

(a mean of 1.8 drugs) than those without this variation (a mean of 1.4 drugs, P=0.016; data not shown). As shown in Table 2, the frequencies of the *eNOS* genotypes were virtually identical to those predicted by the Hardy-Weinberg equilibrium. There were significant differences in the distribution of the *eNOS* genotypes and alleles between the two groups. Table 3 shows odds ratios for CSA in patients with HCM determined by logistic regression analysis. The odds of CSA in patients with the Asp allele was 8.8-fold in the univariate model and 11.8-fold in the multivariate model. In addition, smoking was also a significant independent risk factor for CSA.

Discussion

This study is the first report of an association between the Asp298 variant of the *eNOS* gene and CSA in patients with HCM. Our results suggest that in the Japanese population the Asp298 variant is associated with CSA in patients not only with pure CSA [6] but also those with HCM. Although the functional association between the Glu298 Asp polymorphism and CSA has not been clearly demonstrated, some potential mechanisms have been reported. The *eNOS* wild-type Glu298 and the Asp298 variant are differentially processed in cells [13]. The Asp298 variant is more prone to cleavage by naturally occurring proteases, which cleave the eNOS protein in the region of conservative replacement in the variant forms. Therefore, the Asp 298 variant has a shorter half-life in endothelial cells [13]. In addition, computer analysis has revealed that the Glu298 Asp polymorphism induces a conformational change from an α-helix to a tight turn in the eNOS protein, suggesting that homozygosity for the Asp298 variant may result in a reduction in eNOS activity [6]. Clinical studies have demonstrated that vascular responsiveness is altered in subjects with this variant, as patients with the Asp298 variant have an increased vasoconstrictive response to phenylephrine [14], consistent with decreased eNOS activity.

In this study, the frequency of cigarette smokers was higher in the HCM patients with CSA than in those without CSA, consistent with previous reports [3, 15–18]. Cigarette smoking is a risk factor for CSA in Japanese patients [3, 15, 16] as well as in Western patients [17, 18] who do not have HCM. Kugiyama et al. [19] reported that a deficiency in nitric oxide bioactivity associated with cigarette smoking might contribute to the genesis of coronary spasm. Yoshimura et al. [6] showed the most important risk factor for coronary vasospasm was the Glu298Asp variant followed by cigarette smoking, consistent with our data. In addition, there was a weak association between obstructive HCM and CSA that reached borderline significance in the multivariate regression model (P=0.06).

The frequency of the Asp298 variant in our HCM patients (Table 4) was higher than in the healthy Japanese population [6]. The *eNOS* gene is a modifier gene for HCM rather than the causative gene, it may affect several symptoms such as chest pain and the severity of the disease. Thus, HCM patients with the Asp298 variant may be subject to more significant symptoms, resulting in a population bias. The prevalence of the Asp298 variant in our

Table 4 Frequencies of the *eNOS* gene polymorphism in healthy Japanese. *HCM* Hypertrophic cardiomyopathy

	HCM (n=150)	Yoshimura et al. [6] (n=100)	Shimasaki et al. [30] (n=607)	Hibi et al. [31] (n=357)	Yoshimura et al. [32] (n=345)
Mean age (years)	62	62	56	63	60
Male gender	107 (78%)	48 (48%)	403 (66%)	285 (80%)	177 (51%)
Asp/Asp	3 (2%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)
Asp/Glu	23 (15%)	9 (9%)	80 (13%)	62 (17%)	44 (13%)
Glu/Glu	124 (83%)	91 (91%)	526 (87%)	295 (83%)	301 (87%)
P value (versus HCM)		0.113	0.016	0.025	0.022